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RESEARCH ARTICLE.....

Physico-chemical parameters and microbial identification of flavour effluent

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ABSTRACT..... Flavour effluent produces various pollutants depending upon the process used. The present study deals with the analysis of physicochemical parameters and isolation and identification of microbes (bacteria and fungi) of untreated flavour effluent for a period of 3 months. The results of physico-chemical parameters of untreated flavour effluent revealed that pH was alkaline and other parameters such as electrical conductivity, total suspended solids, total dissolved solids, biological oxygen demand and chemical oxygen demand were found to be beyond the permissible limits of CPCB (1995). The results of microbial analysis showed the presence of 2 types of bacteria-Gram positive cocci, *Staphylococcus aureus* and Gram negative bacilli, *Escherichia coli* and 5 species of fungi which include *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. which were isolated from untreated flavour effluent.

KEY WORDS..... Flavour effluent, Physico-chemical parameters, Microbial identification

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INTRODUCTION.....

Pollution is a gift of rapid industrial and excessive exploitation of natural resources. Pollution is an undesirable change in the physical, chemical or biological characteristics of air, land and water that will harmfully affect the human life (Odum, 1971). The pollution causing adverse effects on physical, chemical and biological factors of water bodies is known as water pollution. Water pollution occurs due to discharge of effluent from different industries such as tanneries, food industry, textile industry etc. (Pandey and Carney, 1998). Flavour Industry (Food Industry) is one of the important industry that cause water pollution. This industry is involved in the production and processing of flavours which provide taste to a large number of goods and beverages such as sweets, meat and soups, beverages, dairy products, etc. which are naturally occurring substances that are produced by synthetic means and artificial substances (Thakur, 2006). Flavorants, more commonly called taste or flavor enhancers are largely based on amino acids and nucleotides. These are typically used as sodium or calcium salts which includes salts of glutamic acid, glycine, gyanylic acid, inosinic acid and 5'- ribonucleotide. Flavours after processing generates effluent which are alkaline in nature with an unpleasant odour, BOD, TSS, TDS. etc. when such flavour effluent discharged into

water bodies affect the physical, chemical and biological characteristics of water and depletes the dissolved oxygen, increased alkalinity, suspended solids etc which are injurious to fish and other aquatic lives. Disposal of such wastes with high pollution load into water course or onto land, with or without prior treatment, creates a great problem in the environment in the vicinity. Hence, it has become essential to treat the effluent to a certain degree prior to its disposal. The treatment of industrial wastes varies with its character, quantity and the nature of receiving media and the dilution available. Pre-treatment of waste water implies removal of floating material, inorganic matter, oil and grease and suspended solids from waste water or conditioning of wastewater for leading the wastewater to a secondary treatment facility through neutralization and or equalization (Kannagi, 2007). Thus the preliminary study was undertaken to analyse physico-chemical parameters and to isolate and identify microbes, both bacteria and fungi present in untreated flavour effluent.

Source and characteristics of micro-organisms, plant growth and their microbial change from dairy wastes from a medium sized effluent was studied and analyzed by Kulkarni (1992). Assessment of physico chemical parameters of effluents from three factories of bareilly district was carried out by Singh *et al.* (1998). Noorjahan *et al*. (2004) reported the characterization of dairy effluent. Biodegradation of crude petroleum using fungi which was isolated from two oil seeds (Melon and Soyabean) was carried out by Adekunle and Oluyode (2005). Physico-chemical characteristics and identification of microbes from untreated brewery effluent have been treated using native fungus *Aspergillus niger* was reported by Kannagi (2007). Bacterial strains isolated from tannery effluent was used to reduce chromium from tannery effluent was studied by Soha and Sohar (2010). Physico-chemical characterization and identification of fungi was carried out by Noorjahan (2014) and reported the presence of 10 fungal species from industrial effluent. Marin *et al.* (2015) carried out the study on Characterization of bacteria and protozoa present in sludge treatment lines of Spanish waste water. He found out that *E. coli* is present in higher level in raw water. Jinhong Zhou*et al.* (2015) identified bacterial and viral pathogens which was focused on their survival or fading in the process of waste water treatment, reclamation and environmental reuse. He reported that *E. coli* and other pathogens was frequently detected which were originated from non-fecal sources also. Kumar *et al*. (2015) studied the Characterization and isolation of fungi for removal of colour from pulp and paper mill effluent, in Meerut. He concluded that fungus has shown to be efficient in the decolonization of the pulp and paper mill effluent and it acts as an alternative treatment for treating the effluents. Erlon *et al*. (2016) carried out the study on physico-chemical and ecotoxicological characterization of slaughterhouse wastewater (SHWW) Resulting from Green Line Slaughter. Based on the ecotoxicological tests, he concluded that the compounds of the SHWW in *natura* were more toxic to microcrustaceans and algae than to bacteria.

Objectives :

– To analyze the physico-chemical parameters of flavour effluent.

– To isolate bacteria and fungi from flavour effluent and

– To identify the bacteria and fungi from flavour effluent.

RESEARCH METHODS.....

Untreated flavour effluent was used as the material in this study. The untreated sample was collected from the point where in all the effluent were discharged together from flavour company situated in Chennai, Tamil Nadu, India, in polythene containers (5 litres capacity). They were brought to the laboratory with due care and stored at 25°C for further analysis. The samples were collected for a period of 3 months from June 2015 to August 2015. The physico-chemical parameters such as Colour, Odour, pH, Electrical Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), of untreated flavour effluent were determined by following the Standard Methods outlined by APHA (1995).

Flavour effluent of about 1 litre was collected in sterile bottles and brought to the laboratory. Isolation and identification of microbes (bacteria and fungi) were carried out on the same day. Untreated flavour effluent was diluted to 10^{-1} using sterile distilled water. 1 ml of the diluted sample was cultured on Nutrient Agar Medium (NA) following pour plate method for bacterial

identification. The cultured Petri plates were incubated at 37°C for 24 to 48 hrs and after the incubation period, the bacterial colonies developed on the medium were observed. Different groups of bacteria present in the water samples were identified by using Gram's staining method. Almost all bacteria can be separated into two large groups called gram positive and gram negative bacteria based on gram's staining technique. Gram positive and gram negative bacteria were identified by following the procedure of Powar and Daginwala (1995).

Bacteria present in the untreated flavour effluent were further identified by biochemical reactions such as catalase and Oxidase test, Indole, Methyl red, Voges proskauer and Citrate utilization (IMViC) test and it is confirmed by doing special tests-coagulase test and EMBA test by following the procedure of Sundararaj (1997).

Untreated flavour effluent was diluted to 10^{-1} using sterile distilled water. 1 ml of diluted sample was cultured on Malt Extract Agar Medium (MEA) following pour plate method for fungal identification. Fungal species developed on the medium was observed. The fungal colonies grown on Malt Extract Agar Medium were subcultured on Potato Dextrose Agar (PDA) slants. The fungi were stained with lactophenol cotton blue and identified using the Manual of Onions *et al.* (1981).

RESEARCH FINDINGS AND ANALYSIS.....

The results of physico-chemical parameters of untreated flavour effluent collected for a period of 3 months (June 2015 to August 2015) are depicted in Table 1. The results of the study revealed that colour of the untreated flavour effluent was brick red and odour was unpleasant. This colour and odour could be due to decomposition of organic or inorganic matter (Singh *et al.*, 1998). A large number of pollutants can impart colour, taste and odour to the receiving water, thereby making them unaesthetic and unfit for domestic consumption (Goel, 1997).

pH of the flavour effluent was found to be alkaline (7.5-7.9). Highly alkaline water if consumed would affect the mucous membrane and may cause metabolic alkalosis The toxicity of certain substances present in water may be enhanced due to their interaction with high or low levels of pH prevailing which may further be detrimental to aquatic organisms (Goel, 1997). Untreated flavour effluent showed higher level of Electrical conductivity (1673-1944 µmhos/cm) than the permissible limits of CPCB (1995), which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Robinson and Stokes, 1959). The composition of solids present in a natural body of water depends on the nature of the area and the presence of industries nearby. High amount of suspended particles were found in the effluent (358-378 mg/lit.) which may have the adverse effects on aquatic flora and fauna and reduce the diversity of life in aquatic system and promote depletion of oxygen and sliting in ponds during rainy season (Goel, 1997). High levels of TDS were found in the effluent (2070-2500 mg/lit.) this may be due to high salt content and also renders it unsuitable for irrigation hence further treatment or dilution of the effluent would be required (Goel, 1997). If the TDS level of water exceeded 500 mg/lit., it becomes unsuitable for bathing and drinking purposes for animals as it could cause distress in cattle and livestock (Singh *et al.*, 1998).

The results of present study revealed high levels of BOD (180-300 mg/lit.) in the flavour effluent due to the presence of considerable amount of organic matter. High BOD levels have also been reported for effluent discharged from tanneries (Kulkarni, 1992) and dairy effluent (Noorjahan *et al.*, 2004). The presence of organic matter will promote anaerobic action leading to the accumulation of toxic compounds in water bodies (Goel, 1997). The present investigation revealed high levels of COD (400-497 mg/lit.) in the effluent. This indicates that the effluent is unsuitable for the existence of aquatic organisms due to the reduction in dissolved oxygen content (Goel, 1997).

The results of isolation and identification of bacteria from flavour effluent is shown in Table 2. Identification of bacteria was carried out by gram staining technique which showed the appearance of violet colour confirmed the presence of gram positive cocci and pink colour indicated the presence of gram negative bacilli.

Gram positive cocci were further subjected to biochemical tests. The results revealed positive for Catalase test and negative for Oxidase test, Indole test gave negative result which showed the absence of tryptophan. Methyl Red test was negative showing the inability of the cocci to oxidize glucose. Negative result was obtained for voges - proskauer test showed the absence of acetoin. Negative result for citrate utilization

was due to the inability to ferment citrate.

Positive result for coagulase test confirmed the presence of gram positive cocci, *Staphylococcus aureus* in untreated flavour effluent.

Gram negative bacilli were further subjected to biochemical tests. The results were catalase positive and oxidase negative, Indole test gave positive result which showed the presence of tryptophan. Methyl red test was positive showing the ability of the bacilli to oxidize glucose. Negative result was obtained for voges - proskauer test showed the absence of acetoin. Negative result for citrate utilization was due to the inability to ferment citrate and presence of green metallic sheen colonies on Eosin Methylene Blue agar (EMBA) was observed. Hence the above results confirmed the presence of gram negative bacilli, *Escherichia coli* in untreated flavour effluent.

Bacteria and fungi are the chief agents for the biodegradation of organic compounds. Yeast, algae and diatoms as well as some higher plants and animals also metabolize a variety of chemicals (Ninnekar, 1992). Microbes especially bacteria act as bio indicator of high polluted effluents as reported by Soha and Sohar (2010). Marin *et al.* (2015) reported that *E.coli* was analyzed as an indicator of the fecal enterobacteria present at high concentrations in the incoming water (Raw water). The presence of these detected micro-organisms may be harmful to health when the effluents (Water and sludge) are reused in agriculture. Jinhong Zhou *et al.* (2015) reported that chlorination was proved to be effective for eliminating all pathogens. In the artificial lake where the product water was stored, fecal coliform was not detected during the study period, but *E. coli* and pathogens were frequently detected, indicating that these bacterial and viral pathogens may be originating from non-fecal sources. Erlon *et al.* (2016) studied the physical, chemical and toxicological characteristics of slaughter house waste water (SHWW) in *natura*

Table 1 : Physico-chemical parameters of untreated flavour effluent collected for a period of 3 months from June 2015 - August 2015

Sr. No.	Parameters	CPCB (1995)	June 2015	July 2015	August 2015
			Untreated	Untreated	Untreated
1.	Colour	Colourless	Brown	Brown	Brown
2.	Odour	Odourless	Unpleasant	Unpleasant	Unpleasant
3.	pH	5.5-9.0	7.7±0.152	7.5±0.1	7.9±0.1
4.	Electrical conductivity (µmhos/cm)	400	1944±1.52	1673±1.52	1702±1.52
5.	Total suspended solids (mg/lit.)	100	358±1	378±2	360±2
6.	Total dissolved solids (mg/lit.)	2100	2070±1.52	2500±1.52	2200±2.08
7.	Biochemical oxygen demand (mg/lit.)	30	300±2	292±2.51	180±1.52
8.	Chemical oxygen demand (mg/lit.)	250	400±2.08	450±1.52	497±1

± = Standard deviation

Table 2 : Biochemical tests of bacterial culture isolated from untreated flavour effluent

Sample	Organism	Gram staining	Catalase test	Oxidase test	Indole test	Methyl-red test	Voges proskauer test	Citrate utilization test	Motility	Special test
Untreated flavour effluent	*Staphylococcus aureus*	Positive Cocci	Positive	Negative	Negative	Negative	Negative	Negative	Non - motile	Coagulase Positive
	Eschericha coli	Negative Bacilli	Positive	Negative	Positive	Positive	Negative	Negative	Motile	EMBA Test

Table 3 : Isolation and identification of different fungi from untreated flavour effluent

Medium	Fungi
Malt extract agar	*Aspergillus* sp
	Mucor sp.
	Penicillum sp.
	Rhizopus sp.
	Trichoderma sp.

collected from a bovine slaughter house and he concluded that the compounds of the SHWW in *natura* were more toxic to microcrustaceans and algae than to bacteria. Sukumaran *et al*. (2008) suggested that the native microbial population present in effluent can be used for biodegradation.

The results of isolation and identification of fungal species is depicted in Table 3. The results revealed the occurrence of 5 species of fungi which include *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. Flavour effluent is rich in organic and inorganic nutrients which would have supported the growth of fungal population (Prabakar, 1999). Noorjahan *et al.* (2003) identified 7 species of fungi in both untreated and industry treated dairy effluents. Adekunle and Oluyode (2005) reported the presence of 21 fungal species in melon and soyabeans. Kannagi (2007) reported the presence of 6 species of fungi in brewery effluent. 6 fungal species were identified in Tannery effluent (Krishna Priya, 2010). The presence of 5 species of fungi in the flavour effluent as reported in the present study has significance in their utility as biological indicators (Rao and Rao, 2000). Kumar *et al.* (2015) concluded that fungus has shown to be efficient in the decolonization of the pulp and paper mill effluent and it acts as a alternative treatment for treating the effluents. Noorjahan (2014) studied degradation of tannery effluent by microbes and concluded that it seems to be most promising technique for 100 per cent untreated tannery effluent. After degradation the treated water could be used for crop cultivation or irrigation and aquaculture purpose.

Aquatic ecosystem is being continuously exploited as a waste disposal system. The aquatic ecosystem is being polluted by the effluent from a number of industries. Flavour industry is one of the important industry that pollutes the aquatic environment. The effluent discharged from flavour industry constitutes a number of pollutants such as BOD, COD, TSS, TDS, etc. These pollutants affect the physico chemical characteristics of the aquatic ecosystem and in turn affect aquatic flora and fauna (Krishna Priya, 2010). In the present investigation, the results of physico-chemical characteristics of the untreated flavour effluent has revealed that it is brick red in colour with unpleasant odour. It is alkaline in nature with high EC, BOD, COD, TDS and TSS and thus the analysis of physico-chemical parameters and microbial identification of untreated flavour effluent for the period of 3 months (June 2015 - August 2015) confirms that the wastewater released from the flavour industry has higher concentration of EC, BOD, COD, TSS, TDS, which surpassed the permissible limits prescribed by CPCB (1995) for discharge of industrial effluent into inland surface water as well as on land for irrigation and it should be treated before its disposal into the environment.

Conclusion :

Flavour effluent is one of the most important water polluters of the environment. It contains large quantity of toxic substances. Hence from the overall results of the above study it can be concluded that the presence of microbes (both bacteria and fungi) indicates the pollutional status of the untreated flavour effluent suggesting that it should be treated before its disposal.

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LITERATURE CITED.....

Adekunle, A.A. and Oluyode, T.F. (2005). Biodegradation of crude petroleum and petroleum products by fungi isolated from two oil seeds (Melon and Soyabean). *J. Environ. Biol.*, **26** (1): 37-42.

APHA (1995). Standard methods for the examination of water and waste water. American Public Health Association, Washingtom, DC. 17th Ed.

CPCB (1995). Status report of groundwater quality in problem areas. Central Pollution Control Board, Delhi, India.

Erlon, Lopes Pereira, Teresa, Cristina Brazil de Paiva and Flávio, Teixeira da Silva (2016). Physico-chemical and ecotoxicological characterization of slaughterhouse wastewater resulting from green line slaughter. *Water, Air, & Soil Pollution*, **227** : 199.

Goel, P.K. (1997). *Water pollution, causes, effects and control*. New Age International (P) Ltd., Publishers, New Delhi, 269.

Jinhong Zhou, Xiaochang C. Wang, Zheng Ji, Limei Xu and Zhenzhen Yu (2015). Source identification of bacterial and viral pathogens and their survival/fading in the process of wastewater treatment, reclamation, and environmental reuse. *World J. Microbiol. & Biotechnol.*, **31**(1): 109–120.

Kannagi, S. (2007). Physico-chemical characteristics and identification of microbes of untreated brewery effluent and its treatment using native fungus *Aspergillus niger*, aquatic plant, water hyacinth – *Eichhornia* sp. and green mussel – *Perna viridis*, M.Sc. Dissertation, University of Madras.

Krishna Priya, E. (2010). Biodegradation of Tannery Effluent using native fungus, *Penicillium* sp. B.Sc., Dissertation University of Madras.

Kulkarni, R.T. (1992). Source and characteristics of dairy wastes from a medium sized effluent on micro-organisms, plant growth and their microbial change. *Life. Sci. Adv.*, **3** :76-86.

Marín, I., Goñi, P., Lasheras, A.M. and Ormad, M.P. (2015). Efficiency of a Spanish wastewater treatment plant for removal of potentially pathogens: Characterization of bacteria and protozoa along water and sludge treatment lines. *Ecological Engg.*, **74** : 28–32.

Ninnekar, H.Z. (1992). Biodegradation of environmental pollutants. *Environ. Biodeg.*, **2**(5): 149-154.

Noorjahan, C.M., Dawood Sharief, S., Nausheen, Dawood and Jamal Mohamed, M. (2003). Isolation and identification of microbes in dairy effluent. Proc. Natl. Conf. On "Envirotech 2003" held at JBAS College for Women, Chennai on 29th and 30th December.

Noorjahan, C.M., Dawood Sharief, S. and Nausheen Dawood (2004). Characterization of dairy effleunt. *J. Indus. Pollut. Cont.*, **20**(1): 131-136.

Noorjahan, C.M. (2014). Physicochemical characteristics, identification of fungi and biodegradation of industrial effluent. *J. Environment & Earth Sci*., **4**(4):32-39.

Odum, E.P. (1971). *Fundamentals of ecology*. 3rd Ed., W.B. Saunders Company, philadelphia, London.

Onions, A.H.S., Allsopp, D. and Egginns, H.O.W. (1981). *Smith's introduction to industrial mycology*, Edward Arnold Publishers Ltd. (7th Ed).

Pandey, G.N. and Carney, G.C. (1998). *Environmental engineering*. Tata McGraw Hill publishing company limited, New Delhi.

Powar, C.B. and Daginwala, H.F. (1995). *General Microbiology*, Meena Pandey Himalaya Publishing House, Bombay.

Prabakar, K. (1999). Studies on Bikoremediation of sugar and distillery effluent. Ph.D. Thesis, University of Madras.

Kumar, Rajesh, Kumar, Rajdeo, Chauhan, Ashish, Kumar, Manoj, Goyal, Manish Kumar and Thakur (2015). Characterization and isolation of fungi for removal of color from pulp and paper mill effluent, Meerut, India. *J. Environ. & Analytical Toxicol.*, **5** (6) : 1-7.

Rao, V. and Rao, V. (2000). Microfungi their diversity and distribution in polluted and non polluted water bodies from an industrial area (Patan cheru) in Hyderabad, Andhra Pradesh, India, *J. Aqua-Biol.*, **15**(11): 112-114.

Robinson, R.A. and Stokes, R.H. (1959). *Electrolyte Solutions*, 2nd (ed.), Academic Press, New York, P.466.

Singh, S.M., Varshneya, I. and Nagarkoti, M. (1998). Assessment of physico chemical parameters of effluents of three factories of bareilly district and their possible effects on grazing animals and cereals. *J. Environ. Biol.*, **19**(3): 271-274.

Soha, Farag and Sohar, Zaki (2010). Identification of bacterial strains from tannery effluent and reduction of hexavalent chromium. *J. Environ. Biol.*, **3** (5):867-872.

Sukumaran, M., Rama Murthy, V., Raveendran, S., Sridhara, G., Netaji S. and Boominathan, M. (2008). Biodiversity of Microbes in Tannery Effluent. *J. Ecotoxicol. & Environ. Monitoring*, **18** (4) : 313-318.

Sundararaj, T. (1995). Microbiology Laboratory Manual. IBMS, University of Madras, Tharamani, Chennai, 48-62.

Thakur, I.S. (2006). *Industrial Biotechnology: Problems and Remedies*, I-K. International Pvt. Ltd., New Delhi .142-183.

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